



Ciência Rural

ISSN: 0103-8478

cienciarural@mail.ufsm.br

Universidade Federal de Santa Maria  
Brasil

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Ciência Rural, vol. 45, núm. 2, febrero, 2015, pp. 200-205  
Universidade Federal de Santa Maria  
Santa Maria, Brasil

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## Physiological and phytosanitary potential of rocket seeds

### Potencial fisiológico e fitossanitário de sementes de rúcula

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#### ABSTRACT

The objective of this study was to evaluate the physiological and sanitary quality of seeds of rocket; the research was done at the Laboratory of Seed Analysis and greenhouse of the Department of Plant Science, Federal University of Pelotas (UFPEL). Four lots of the cultivar "Antonella" were tested for following features: initial and final moisture content, germination rate, first count of germination, accelerated aging with saline solution, dry matter contents, seedling shoot and root length, emergence speed index, emergence of seedlings in substrate, electrical conductivity and sanitary condition. A completely randomized design with four replications was used for all tests done and means were compared by Tukey test ( $P \leq 0.05$ ). For all tests performed it was concluded that despite changes in the ranking of the best lots, there was agreement regarding the indication of the inferiority of the lot 3 in all tests and it was also observed that the incidence of fungi associated with seeds of rocket interfere with the physiological quality of the lots.

**Key words:** *Eruca sativa* L., vigour tests, vegetable seeds.

#### RESUMO

O objetivo nesta pesquisa foi avaliar a qualidade fisiológica e sanitária de sementes de rúcula. O trabalho foi conduzido no laboratório e em casa de vegetação do Departamento de Fitotecnia da Universidade Federal de Pelotas (UFPEL). No ensaio, foram utilizados quatro lotes de sementes de rúcula da cultivar 'Antonella', sendo efetuados os seguintes testes: grau de umidade inicial e após o envelhecimento acelerado, germinação, primeira contagem de germinação, envelhecimento acelerado com solução salina, massa seca da parte aérea e de raiz de plântulas, comprimento da parte aérea e de raízes de plântulas, índice de velocidade de emergência, emergência de plântulas, condutividade elétrica e teste de sanidade, em delineamento experimental inteiramente casualizado, com quatro repetições, sendo que as médias foram comparadas pelo teste de Tukey ( $P \leq 0,05$ ). Apesar das alterações na classificação dos melhores lotes, nos testes

analisados, verificou-se concordância quanto à indicação da inferioridade do lote 3 em todas as avaliações. Verificou-se que a incidência dos fungos associados às sementes de rúcula interfere na qualidade fisiológica dos lotes.

**Palavras-chave:** *Eruca sativa* L., testes de vigor, sementes de hortaliças.

#### INTRODUCTION

The rocket plant (*Eruca sativa* L.) is an herbaceous annual vegetable of low-height, usually 15 to 20cm height, which belongs to the family of Brassicaceae and is native to the Mediterranean Region and West Asia (FILGUEIRA, 2008). It is a vegetable with a growing increase in consumption in Brazil (CAMARGO FILHO & MAZZEI, 2001) and the high cost of seeds and hand labor involved in its cultivation requires studies that relate the quality of seeds to seedling emergence, in order to determine the cause of crop losses (GOULART & TILLMANN, 2007).

The physiological quality of seeds used by farmers is a major factor to be considered for the deployment of the crop and there is consensus among researchers, technologists and producers of the importance of vigor and the need to evaluate it (KOLCHINSKI et al., 2006) because high vigor seed lots show rapid and uniform seedlings emergence, which are essential to the formation of well-developed plants, thus contributing to increase the final yield of the crop and quality of the commercial product (MARCOS

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FILHO & NOVENBRE, 2009; DALIL et al., 2010). In addition to the use of high quality seeds for the establishment of uniform and vigorous crops, there is a concern over the presence of pathogens associated with seeds because contaminated seeds are responsible for the introduction of disease in early stages of the culture of crops, reflected in reduced yields, increased costs of production, and allow the introduction and maintenance of high levels of inoculum in the field (MACHADO, 2000; ZORATO & HENNING, 2001; LUZ, 2003).

The presence of pathogens in the seeds, regardless of their transmissibility can affect the vigor and field performance and fungi associated with seeds may be responsible for transmitting diseases to shoot and root systems and decrease the physiological quality of seeds and increase the risk of seedling death (LUCCA-FILHO, 1995). In this context, high vigor seed and free of pathogens are essentials (MACHADO, 2000) and the physiological potential should be determined by assessing germination and vigor of the seed lots because seed vigor has several characteristics associated with the performance of the seed lots, being able to identify results of their behavior during storage or in the field (MARCOS FILHO, 2005).

Several tests of vigor for vegetable seeds have been published, but the availability of information on the evaluation of vigor of rocket seed is still quite limited, but the following works can be cited: the efficiency of the accelerated aging test was explored by RAMOS et al. (2004) and GOULART & TILLMANN (2007) have evaluated the employability of the controlled deterioration test and the electrical conductivity and STEINER et al. (2010) has studied seed germination under different temperatures. In order to contribute to increase information about vigor and seed health of vegetable, this study aimed to evaluate the efficiency of different tests to determine the physiological quality and health of different lots of rocket.

## MATERIAL AND METHODS

The work research using seeds from four seed lots of rocket cultivar 'Antonella' (from Isla Seed Company) were subjected to following tests and assessments:

**Determination of moisture content:** the moisture content of the seed was assessed before and after the accelerated aging test and the test was conducted in an oven at  $105 \pm 3^\circ\text{C}$  for 24h, in accordance with the Rules for Seed Analysis - RAS (BRASIL, 2009), using two samples of 4 g seeds

for each lot and the results were expressed as mean percentage weight loss per lot.

**Germination test:** was conducted at  $20^\circ\text{C}$  with 200 seeds per seed lot (four replicates of 50 seeds); the seeds were distributed on two sheets of paper, previously moistened with distilled water in an amount equivalent to 2.5 times the mass of paper and placed inside transparent plastic boxes (11.5x11.5x3.5cm) with lid. Evaluations were conducted at four and seven days after sowing and the results expressed in percentage of normal seedlings (BRASIL, 2009).

**First count of germination:** was made in conjunction with the germination test, by computing the percentage of normal seedlings on the fourth day after sowing (BRASIL, 2009).

**Accelerated aging with NaCl solution:** a total of 200 seeds (four replicates of 50) per lot were distributed over an aluminum screen placed inside a plastic box (gerbox) containing 40ml of NaCl solution, maintained at  $41^\circ\text{C}$  for 48 hours. This solution was prepared by adding 40g of NaCl in 100ml of water, establishing an environment with relative humidity of 76%, according to the procedure proposed by JIANHUA & MCDONALD (1997). After this period, the seeds were put to germinate following the methodology used in the germination test described above and the percentage of normal seedlings was assessed on the fourth day after sowing, as described by GOULART & TILLMANN (2007).

**Dry matter contents:** the same twenty normal seedlings used for root and shoot length measurement were also used for dry matter content measurement. The samples were dried in a forced-air oven at  $60^\circ\text{C}$  for 72h, cooled in a desiccator, and weighed.

**Seedling shoot and root length:** was conducted at  $20^\circ\text{C}$  with 80 seeds per seed lot (four replicates of 20 seeds); normal seedlings were taken from each replication for measuring the root and shoot length and the mean values were recorded in cm.

**Emergence Speed index:** the number of emerged seedlings was counted daily until it reached a constant level (a period of 21 days). For each replication, the emergence speed index was calculated by summing the daily ratios of the number of emerged plants to the number of days from sowing using the formula described by MAGUIRE (1962).

**Seedling emergence in substrate:** a total of 200 seeds (four replicates of 50) per lot were distributed in polystyrene trays with 200 cells filled with commercial substrate Plantmax®. Evaluations were performed at 12 days after sowing, computing

the normal seedlings and the results expressed as percentage of normal seedlings emerged.

Electrical conductivity: the seeds were soaked in 200mL plastic cups containing 50mL of deionized water and the readings were taken at 2, 4, 6, 8 and 24 hours and the test was done with four replications of 50 seeds per seed lot and values were expressed in  $\mu\text{S cm}^{-1} \text{ g}^{-1}$ .

Seed health testing: the methodology of the paper filter or blotter test was used, eight sub samples of 25 seeds per seed lot were placed in plastic boxes containing three filter papers moistened with -0.5MPa of osmotic potential of polyethylene glycol PEG 6000 (VILLELA et al., 1991). The seeds were incubated at 25°C for seven days under a photoperiod regime of 12 hours light and 12 hours of darkness and after the incubation period the seeds were examined individually for fungal growth using a stereomicroscope, and the incidence of pathogenic fungi was expressed as percentage seeds infected.

The experimental design was completely randomized layout and in the statistical procedure, analysis of variance was performed separately for each test and the means of the lots were compared by Tukey test at 5% probability. The conductivity test was analyzed in a factorial of 4 lots x 5 seed imbibition periods and to seed health test was not analyzed by statistical test.

## RESULTS AND DISCUSSION

The initial moisture content of the seeds from the four seed lots of rocket ranged from 5.5-7.5% (Table 1), this is within the maximum range of two percentage points accepted for guarantee credibility, according to MARCOS FILHO (1999). When analyzing the initial quality of the lots, it appears that they had a high germination percentage

ranging from 86 to 93%, which is higher than the minimum of 80% established for seed trading. However, in many cases a high germination percentage does not necessarily mean that all of these lots have a high vigor, since the germination test is conducted under favorable conditions.

The germination test sorted the lots at different levels of vigor, pointing to the low grade in physiological quality of lot 3, differing from lots 1 and 2, but being equal to lot 4. The germination test for other kinds of vegetables, such as zucchini (*Cucurbita pepo* L.) and zucchini cv. Piramoita, also managed to rank seed lots (CARDOSO, 2003). These authors demonstrated that for these species the germination test is a good indicator of physiological quality. Regarding seeds of cucumber (*Cucumis sativus* L.), BHERING et al. (2000) concluded that although the germination test proves to be a good indicator of physiological seed quality, it does not guarantee a high field emergence of lots with higher germination percentages.

The first count test separated the lots regarding their physiological quality; the seeds from lot 1 and 2 showed higher values, whereas lots 3 and 4 showed the worst performance. Similar results were obtained by CARDOSO (2003) with squash seeds, in this work, the lots were only sorted in high and low physiological potential. In cucumber and lettuce, the first count of germination has been routinely used to obtain preliminary information on the physiological potential of seed lots (BHERING et al., 2000).

The accelerated aging test was more sensitive than germination to detect differences in seed vigor and this test also confirmed the poor quality of the third lot and good quality of lots 1 and 2 and lot 4 was found to be intermediate quality (Table 1). In this study, the seed lots 1 and 2 had the lowest levels of water after the accelerated test, whereas, the highest values occurred in seeds of lot 3

Table 1 - Initial moisture content of the lots (IMC), moisture content after accelerated aging (MCA), percentages for germination test (G), first count of germination (FCG), accelerated aging with NaCl-76% (AA), root and shoot dry matter (DM), root length (RL), shoot length (SL), emergence speed index (ESI), emergence of seedlings in substrate (SES) of the different lots of rocket seeds.

----- Physiological characteristics -----										
Lots	IMC	MCA	G	FCG	AA	DM	RL	SL	ESI	SES
	%					-----cm-----			%	
1	5.500	14.400	93 a	88 a	81 a	0.020 a	4.2 a	2.6 a	13.1 a	95 a
2	5.500	12.700	92 a	91 a	80 a	0.020 a	4.1 a	2.5 a	13.3 a	96 a
3	7.500	17.000	86 b	81 b	45 c	0.014 b	1.7 c	1.6 b	10.6 c	88 b
4	5.518	16.550	88 ab	80 b	61 b	0.019 a	3.0 b	2.7 a	12.2 b	92 ab
CV(%)	-	-	6.7	9.0	12.01	11.79	14.75	19.1	6.7	5.5

Means followed by the same letter in a column do not differ in level of 5% probability by the Tukey test.

and 4. This high water content may have influenced the seed germination in the aging test because seeds with low water content showed higher germination rates.

These results are similar to those observed by BHERING et al. (2000) and BARROS et al. (2002) that also found the efficiency of accelerated aging test using the period of exposure of 48 hours and 41°C for stratification of the seed lots of cucumber and zucchini respectively. Furthermore, another study conducted with seeds of rocket by GOULART & TILLMANN (2007) has found that the accelerated aging test was efficient for evaluating quality of rocket seeds because this test was enable in demonstrating vigor differences between lots. The ability of the accelerated aging test to evaluate seed vigor was also observed in seeds of watermelon (BHERING et al., 2003), in this work they showed that the accelerated aging test with the use of salt solution promotes less drastic effects for achieving the lower water content and the degree of deterioration of the seeds is attenuated compared to the traditional method (when only water is used).

With the dry mass of seedlings assessed in days after emergence of seedlings substrate, two levels of physiological quality could be distinguished (Table 1). The seedlings from lots 1, 2 and 4 showed the highest content of dry mass, whereas, the seedlings from third lot had the lowest content of dry mass of shoot and root. In this test, the principle is that seeds whose normal seedlings have higher average contents are considered more vigorous. The flux of mass from storage tissue to the embryonic axis during seed germination is intense, resulting in seedlings with higher weights due to higher accumulation of material (NAKAGAWA, 1999).

The root of seedlings from the lots 1 and 2 were significantly higher than lots 3 and 4, however,

the dry mass of seedlings distinguished the lots in two levels as was observed for the shoots, in the other words, the seedlings from seed lots 1, 2 and 4 were statistically reliable higher than those of lot 3 (Table 1) and in both assessments, the third lot is considered to be of low physiological quality. According to NAKAGAWA (1999) differences in length of seedlings are most often quite visible; however, it is important to separate the more vigorous by numeric values. For this, determining the average length of normal seedlings or parts of them is performed in order that the samples which have the highest average values are more vigorous. BALBINOT & LOPES (2006), working with carrot seeds studied the relationship between seed vigor and length of seedlings.

The speed of emergence index has also showed sensitivity to rank lots of different physiological potential as a significant difference between the averages assessed (Table 1). It is important to emphasize, seed lots with greater physiological potential, mainly by higher rates of emergency speed, are important to obtain seedlings that remain a shorter time subject to adverse conditions, such as the presence of fungi that promote damping off and also by obtaining the earliest and most uniform seedlings (CASAROLI et al., 2006).

A significant interaction between time of seed imbibed and lots was observed (Table 2) and the electrical conductivity test also allowed differentiating levels of quality among seed lots of rocket showing in all imbibing periods that the test also identified the lot 3 as the less vigorous in relation to lots 1, 2 and 4. In other studies with carrot seeds (ANDRADE et al., 1995) and also with rocket seeds (ALVES & SÁ, 2009), promising

Table 2 - Electrical conductivity test with soaking 50 seeds in 50ml of distilled water for periods of 2, 4, 6, 8 and 24 hours from four rocket seed lots.

Lots	Imbibing periods ( $\mu\text{S cm}^{-1} \text{ g}^{-1}$ )				
	2	4	6	8	24
1	5.71 aA	8.20 aB	9.74 aC	10.76 aC	17.95 aD
2	8.00 abA	9.06 aB	11.83 bC	12.86 bC	19.64 bD
3	8.99 cA	11.85 bB	14.44 cB	16.00 cC	25.33 cD
4	7.14 bA	9.01 aB	10.82 abB	11.9 abC	18.94 abD
CV			10.8		

Means followed by the same letter in lower case in a column and capital letter on a line do not differ (Tukey test at 5% probability).

results were found in assessments after four hours of imbibing. In this present study, all imbibing periods were effective in the classification of the lots and this test separated the lots by their quality levels. When the conductivity values are analyzed over the different periods, there is a lot like being the best quality with the system more intact cell membranes or with greater capacity to restore its integrity, during hydration, the less leaching of electrolytes, indicating a greater vigor, as suggested by studies in other crops (DIAS et al., 2006), confirming the results observed in other tests.

The results of the germination test obtained in laboratory are exactly in line with those obtained for the emergence of seedlings in the greenhouse using substrate (Table 1). Thus, differences in quality found in the lots 1 and 2 and 4, were confirmed in two tests, which exhibit the best physiological conditions, obtaining the highest percentage of emergence of the seedlings and lot 3 showed the lowest results. The vigor classification correspondence between germination test in the laboratory and seedling emergence of the lots may be due to the emergence of seedlings had been done in the greenhouse using as a support an inert substrate. This fact contributed to maintain the temperature and sufficient moisture for good seeds germination; in addition the substrate was absence of pathogen.

The higher incidence of fungi was observed for all lots belonging to the genera *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. However, the incidence varied between the different lots (Table 3) and the fungi found in this study can be classified into fungi that are transmitted to the seedlings and plants by seeds, such as *Fusarium* spp.,

*Alternaria* spp. and *Cladosporium* spp. and storage fungi, which are include other genera cited above.

The lot 3 had a higher incidence of fungi on seeds, this may explain the low viability and vigor found in that lot, and it showed that the high incidence of fungi limited the performance of this lot. In this paper was observed that the lots with a lower incidence of fungi, in general, showed higher values of seed germination and vigor. Thus, the sanitary quality of seeds can also influence the seeds vigor and the emergence of seedlings, and the sanity test should be performed to verify the quality of a seed lot produced.

## CONCLUSION

All vigor tests used in this study were effective to separate the seed lots in vigor levels and the tests showed lot 3 as the worst physiological quality. The incidence of fungi associated with seeds of rocket interferes with the physiological quality of the lots.

## ACKNOWLEDGEMENTS

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship for the first author and Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq) for the financial support for this work and we also wish to thank Dr. Pieter Kastelein from Wageningen University and Research Centre, Plant Sciences Group for making useful suggestions for this paper.

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Table 3 - Percentage of fungi obtained in the sanity test of the four rocket seed lots.

Fungi	Lot 1	Lot 2	Lot 3	Lot 4
<i>Aspergillus</i> spp.	4.5	18.3	21	20
<i>Penicillium</i> spp.	0.3	12.33	8.66	6.33
<i>Fusarium</i> spp.	3.2	17.33	37.66	7.33
<i>Rhizopus</i> spp.	1.9	7	8.7	4.3
<i>Curvularia</i> spp.	0	0.3	0.66	0
<i>Cladosporium</i> spp.	0	0.6	2.6	0
<i>Nigrospora</i> spp.	0	0	1	0
<i>Epicoccum</i> spp.	0	0	0	0
<i>Stemphylium</i> spp.	0	0	0	0
<i>Alternaria</i> spp.	0.5	0.3	10	0
<i>Bipolaris</i> spp.	0	0	1	0
<i>Trichoderma</i> spp.	0	0	1.6	0
<i>Chaetomium</i> spp.	0	0	0	0.66

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